



# United States Air Force Research Laboratory

## The Effect of Jet Fuels on the Skin Irritation and Neuropeptide Release

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## TECHNICAL REVIEW AND APPROVAL

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The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

## FOR THE DIRECTOR

//SIGNED//

**MARK M. HOFFMAN**  
Deputy Chief, Biosciences and Protection Division  
Air Force Research Laboratory

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## **FINAL REPORT FOR USAF/AFMC GRANT**

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**List of Manuscripts Published / Submitted:**

1. Mandip Singh\*, S. Yim, K. Ball, N. Kanikkannan and R. J. Babu, Skin absorption, permeation and irritation potential of various chemicals of JP-8 (studied as neat chemicals) in hairless rats, Presented in the Annual JP-8 Jet-Fuel Exposure and Health Effects Symposium held at the university of Arizona, Tuscon, May 15 – 17, 2002.
2. Mandip Singh\*, R. J. Babu, A. Chatterjee, S. Yim, and A. Kundra, Skin permeation, distribution, irritation and molecular responses of various chemicals of JP-8 (studied as neat chemicals) in hairless rats. Presented in the Annual JP-8 Jet-Fuel Exposure and Health Effects Symposium held at the university of Arizona, Tuscon, May 14 – 16, 2003.
3. Kanikkannan N, Locke BR, Singh M., Effect of jet fuels on the skin morphology and irritation in hairless rats. Toxicology, 175 (2002) 35-47.
4. Mandip Singh\*, R. J. Babu, A. Chatterjee, S. Yim, and A. Kundra, Skin permeation, distribution, irritation and molecular responses selected aliphatic hydrocarbons in hairless rats (Manuscript Under Preparation).
5. Mandip Singh\*, R. J. Babu, A. Chatterjee, S. Yim, and A. Kundra, Skin permeation, distribution, irritation and molecular responses of benzene and xylene hairless rats (Manuscript Under Preparation).

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3. Sokha Yim (Undergraduate student)
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## TECHNICAL REPORT

### 1. Skin penetration and absorption into different layers of skin of selected chemicals of JP-8 (Nonane, Dodecane, Tetradecane, Benzene and Xylene) using hairless rat skin

Excised Hairless rat skin (CD® (SD)Hr.Bi, Male rats) was used for permeation and absorption studies. The studies were conducted on Franz diffusion cells using 6% Brij in normal saline (37°C) as the receptor medium which was stirred with a magnetic bar at 600 rev. / min. Nonane, dodecane, tetradecane, benzene and xylene (0.5ml) spiked with 2.5  $\mu$ Ci of respective radiolabeled chemical was placed in the donor compartment. The receptor samples were analyzed by Liquid scintillation counting. The cumulative amount of chemical permeated was plotted against time. The slope of linear portion of the curve ( $\text{mg}/\text{cm}^2/\text{hr}$ ) was determined. For absorptions studies, the skin after defined exposure period was taken out from the diffusion cell. The stratum corneum was removed by tape stripping with Transpore® tape. The underlying tissue was sectioned with Cryotome (Thermo-Shandon, 620 Electronic) into epidermis and dermis. The  $^{14}\text{C}$  and  $^3\text{H}$  samples were counted on a scintillation counter (1219 Reckbeta, LKB Wallac). The amount of chemical remaining in the skin was expressed in  $\text{mg} / \text{g}$  of the tissue.

Fig. 1: Permeation profiles of benzene and xylene through hairless rat skin in vitro

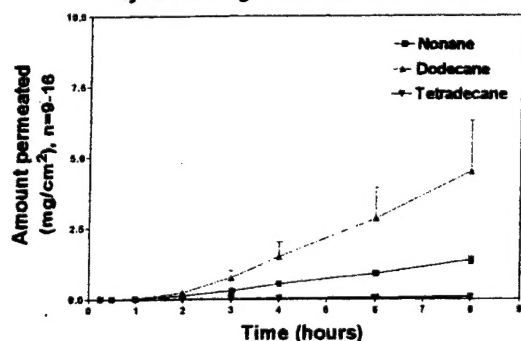


Fig. 2: Permeation profiles of benzene and xylene through hairless rat skin in vitro

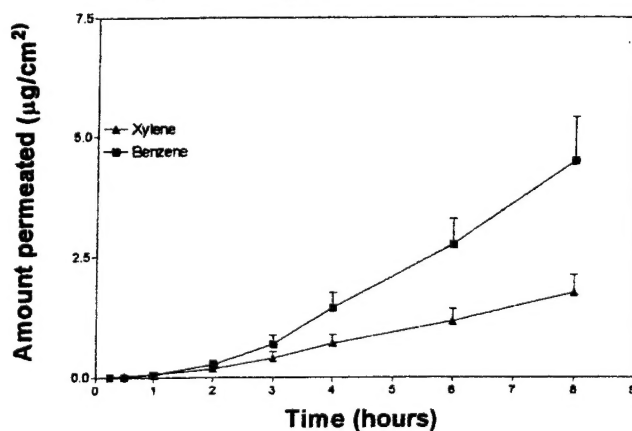


Fig. 3: Absorption of nonane and dodecane into different layers of hairless rat skin in vitro

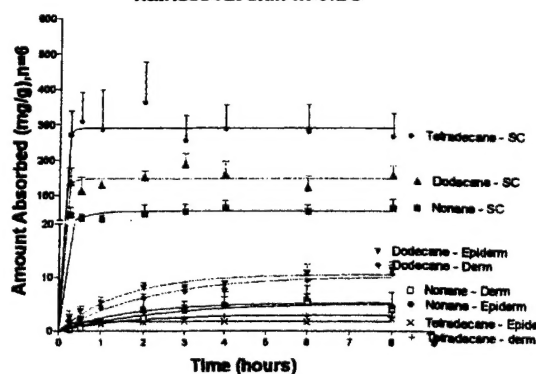
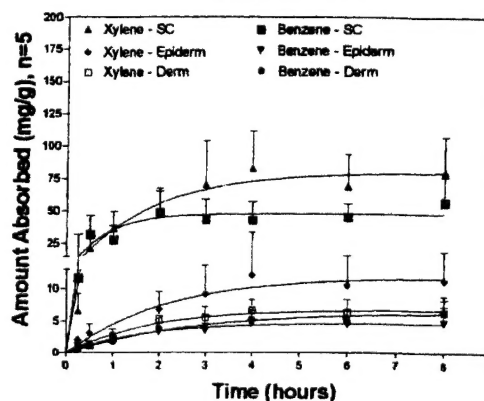


Fig. 4: Absorption of benzene and xylene into different layers of hairless rat skin in vitro



## Results:

- Parabolic relationship between the chain length of the aliphatic chemical and its permeation rate was observed. Tetradecane showed very low permeation, probably due to its high log octanol-water  $K_p$  ( $>7.0$ ) (Fig. 1)
- The permeation rate of aromatic chemicals decreased with increase in the molecular weight (xyl=106.17, benz=78.12) or log  $K_p$  (xyl=3.12, benz=2.13) (Fig. 2)
- The skin retention pattern of the aliphatic chemicals in SC indicates the depot formation according to their lipophilicity. The retention increased with increase in the  $K_p$  of chemicals (Fig. 3).
- However, in epidermis and dermis, which are relatively hydrophilic regions, the retention decreased with increase in  $K_p$ , (excepting dodecane)
- The retention pattern of different chemicals (in epidermis and dermis) was dodecane > nonane > tetradecane, similar to the results of their permeation rates.
- The retention of aromatic chemicals in increased with increase in the lipophilicity in all the skin layers (Fig. 4).
- An inverse correlation between the skin retention of chemicals and their permeation rates was observed.

## **2. Effect short-term occlusive and prolonged unocclusive exposure of selected chemicals of jet fuel on the skin barrier function and skin irritation in hairless rats.**

Hairless rats (CD® (SD)Hr.Bi, Male) were used for occlusive and unocclusive exposure studies. The temperature of the room was maintained at  $23 \pm 1^\circ\text{C}$  and the humidity was maintained at 35-45% RH. The control and treatment areas ( $\sim 3\text{ cm}^2$ ) were marked on the dorsal surface of the rat. For occlusion studies, nonane, dodecane tetradecane, benzene and xylene (230  $\mu\text{L}$  each) were placed in the Hill top chamber® (surface area  $1.04\text{ cm}^2$ ) and affixed over the center of the marked treatment sites for 1 hr. The control site was affixed with a Hill top chamber® without any solution. Measurements of transepidermal water loss (TEWL) and skin capacitance (moisture content) were taken for all the treatment and control sites before application and at intervals up to 7 days after removal of the patches. For unocclusive conditions, the chemicals were applied at 15 $\mu\text{L}$  every 2 hours for 5 days. TEWL and skin moisture content at the treatment and control sites was measured using Tewameter TM 210 (Courage +Khazaka, Koln, Germany) and Corneometer CM 825® (Courage +Khazaka, Koln, Germany), respectively. The skin irritation (erythema and eczema) was evaluated by visual scoring by a modified method of Draize and coworkers.

## Results:

The effect of occlusive application of various chemicals on the skin irritation and barrier function is shown in Figs. 5-7. The skin irritation data correlated well with TEWL data. Dodecane, which was absorbed in highest levels in various layers of skin, caused minimum effect on skin barrier function and irritation under occlusive conditions. The flux of nonane was lowest of all chemicals studied and showed higher TEWL values and skin irritation than xylene and benzene. The effect of unocclusive application of various chemicals on the skin irritation and barrier function is shown in Figs. 8-10. All the chemicals showed significantly higher irritation scores, TEWL and moisture content under unocclusive conditions. Short-term occlusion of skin (1hour,  $230\text{ml}/\text{cm}^2$ ) with various chemicals showed a very high effect on skin barrier function and



irritation than unocclusive condition for prolonged exposures ( $14 \text{ ml/cm}^2$ , every 2 hours for 5 days). The skin barrier function (as indicated by high TEWL) and erythema scores did not return to base line even at the end of 7 days study with short-term occlusive condition.

Fig. 5: Scores of erythema upon occlusive application of various chemicals in hairless rats

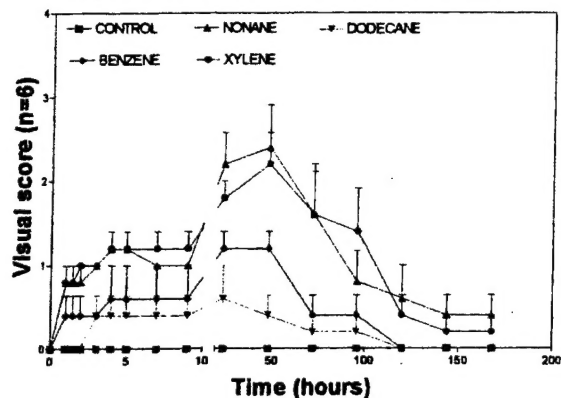


Fig. 8: Scores of erythema upon unocclusive application of various chemicals in hairless rats

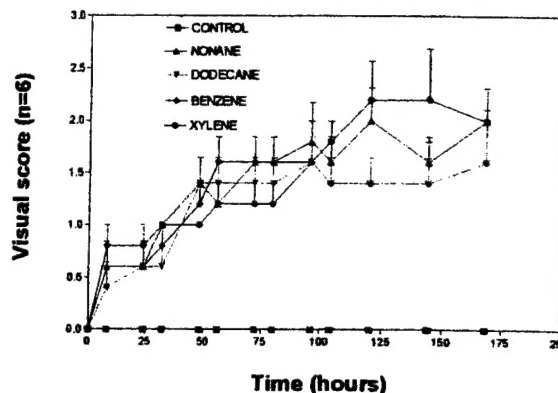


Fig. 6: Effect of application (occlusive) of various chemicals on the TEWL in hairless rats

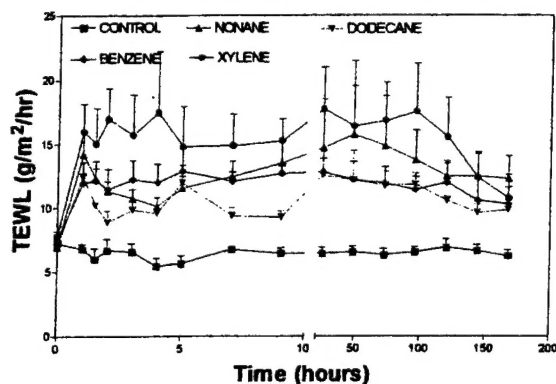


Fig. 9: Effect of application (unocclusive) of various chemicals on the TEWL in hairless rats

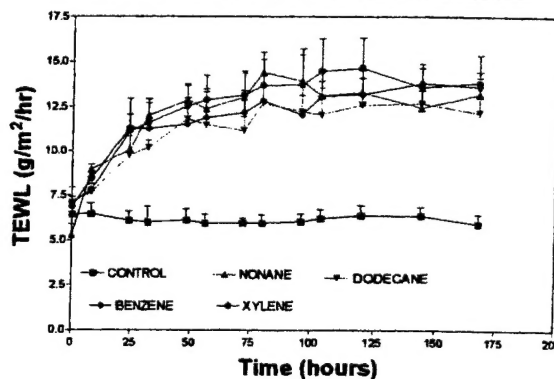


Fig. 7: Effect of application (occlusive) of various chemicals on the skin moisture content in hairless rats

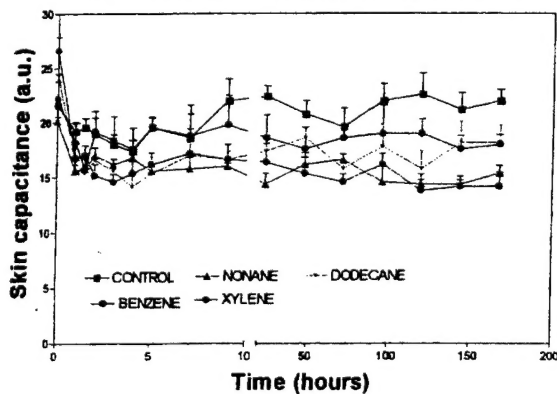


Fig. 10: Effect of application (unocclusive) of various chemicals on the skin moisture content in hairless rats

